killed and analyzed for body calcium at about one year of age, with the results shown in table 1.

TABLE 1

COMPARISON OF CALCIUM CONTENTS OF ADULT RATS FED BASAL DIET 16 ALONE OR WITH THE ADDITION OF 60 GM. PER WEEK OF POULTRY MEAT (DIET 16 P 10)

	FROM DIET 16	FROM DIET 16 P 10
Males (litter mates)		
Age, days	290	290
Weight, g.	295	314
Body calcium, g.	1.87	1.76
Body calcium, %	0.67	0.60
Females (average of 3 in each case)		
Age, days	354	348
Weight, g.	199	221
Body calcium, g.	1.99	1.89
Body calcium, %	1.08	0.93

It will be seen that at full adulthood the animals which had received the protein-enriched diet were still ahead in body weight and behind (their controls) in body calcium. This is the more noteworthy in that the animals which had received the basal diet only contained both larger amounts and higher percentages of calcium in their bodies.

The larger number of the animals which were given this dietary enrichment are being continued, as are their controls, in the hope of carrying the comparison throughout their complete life histories.

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[1] Sherman, H. C., and Pearson, C. S., *Proc. National Acad. Sci.* **33**, 264–266 (1947).

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*DEPLETION MUTATION IN SACCHAROMYCES**

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An α mating type galactose-, maltose-fermenting haplophase segregant of *Saccharomyces cerevisiae* was subjected to mustard gas treatment by Tatum and Reaume (Tatum and Reaume, in ms.) and produced an adenine-dependent mutant with pink colonies. The symbol ad(P) indicates the adenine-dependent variant producing pink colonies; ad indicates the same allele carried by a white phenotype. The symbol AD indicates the dominant allele; no secondary symbol is necessary for

these are always white. The adenine-dependent pink (ad(P)) culture was shown to be a gene mutation by hybridizing it with an adenine-independent white (AD) haplophase of *S. cerevisiae*.

	MATING		HYBRID	SEGREGANTS			
	Haploid	× haploid	Diploid	Haploid	Haploid	Haploid	Haploid
Gene	ad(P)	× AD	ad(P)/AD	ad(P)	ad(P)	AD	AD
Color	Pink	× White	White	Pink	Pink	White	White

Forty-six asci were dissected from the white hybrid and in 42 asci, two white and two pink cultures arose from each ascus, proving that gene mutation was involved (table 1). In these 42 asci the pink cultures were adenine-dependent and the white cultures were adenine-independent.

TABLE 1

GENETICAL ANALYSIS OF ASCI FROM VARIOUS CROSSES INVOLVING PINK AND WHITE COLOR, AND ADENINE AND METHIONINE SYNTHESIS

MATINGS	NO. ASCI	SEGREGANTS A	B	C	D	COLOR
ad(P)MET × AD MET	42	ad(P)	ad(P)	AD	AD	2 pink:2 white
pink white	1	ad(P)	ad	AD	AD	1 pink:3 white
	2	ad	ad	AD	AD	0 pink:4 white
	1	ad(P)	ad(P)	ad(P)	AD	3 pink:1 white
ad(P)MET × AD met	7	ad(P)MET	ad(P)MET	AD met	AD met	2 pink:2 white
pink white	5	ad met	ad met	AD MET	AD MET	0 pink:4 white
	23	ad(P)MET	ad met	AD met	AD MET	1 pink:3 white
ad met × AD MET	7	ad(P)MET	ad(P)MET	AD met	AD met	2 pink:2 white
white white	5	ad met	ad met	AD MET	AD MET	0 pink:4 white
	27	ad(P)MET	ad met	AD met	AD MET	1 pink:3 white
ad(P)MET × ad(P)MET	6	ad(P)MET	ad(P)MET	ad(P)MET	ad(P)MET	4 pink:0 white
pink pink						
ad met × ad met	3	ad met	ad met	ad met	ad met	0 pink:4 white
white white						
ad(P)MET × AD MET	7	ad(P)MET	ad(P)MET	AD MET	AD MET	2 pink:2 white
pink white						
ad MET × ad(P)MET	11	ad(P)MET	ad(P)MET	ad(P)MET	ad(P)MET	4 pink:0 white
white pink						
ad MET × ad(P)MET	10	ad(P)MET	ad(P)MET	ad(P)MET	ad(P)MET	4 pink:0 white
white pink						
ad MET × ad met	8	ad(P)MET	ad(P)MET	ad met	ad met	2 pink:2 white
white white						
ad MET × AD met	1	ad(P)MET	ad(P)MET	AD met	AD met	2 pink:2 white
white white	5	ad(P)MET	ad met	AD met	AD MET	1 pink:3 white

Three asci produced fewer than the expected number of pink segregants. One contained three white and one pink culture; the pink segregant and one white culture were adenine-dependent. Two asci produced 4 white cultures; two were adenine-dependent and two were adenine-independent. These exceptional adenine-dependent white cultures will be discussed in detail below.

From one ascus more than the expected number of pink cultures was

obtained (three pink and one white culture) and the three pink segregants were adenine-dependent. Most of the adenine-dependent cultures adapt after four days and grow in the adenine-deficient medium. The differences in growth in adenine-deficient and adenine-containing medium are generally diagnostic on the second and third days; but growth of the so-called adenine-dependent cultures in the adenine-deficient medium becomes fairly dense on the sixth and seventh day. The extra pink culture may have been slow in adapting to adenine synthesis due to some other deficiency.

The hybrid was also heterozygous for mating type, galactose-, maltose- and melibiose-fermentation and there were no exceptions of these characters to Mendelian segregation.

The hybrid from which the 46 asci were analyzed was homozygous for genes controlling the synthesis of methionine, so that relatively adequate amounts of methionine were available to all four segregants. Tatum and Reaume also discovered a methionine-dependent mutant produced by mustard gas treatment and this gene was introduced into the stock by a series of matings. A hybrid heterozygous for adenine-dependence and methionine-dependence was produced. Analysis of 74 asci from the reciprocal crosses ad(P) MET × AD met (pink × white) and ad met × AD MET (white × white) revealed that the development of pink color required methionine. The adenine-dependent *white* cultures were also methionine-dependent and all the adenine-dependent *pink* cultures were methionine-independent proving that methionine was required for the development of the pink color.

I have pointed out that a doubly heterozygous hybrid produces only three kinds of asci and that the frequency of these three types can be used to detect linkage of either of the genes with each other or with their respective centromeres. The data in table 1, show that the two reciprocal matings, one producing 35 and the other producing 39 asci, both follow the same pattern and the three kinds of asci are present with a total frequency of 14:10:50, which is statistically equivalent to a 1:1:4 ratio, proving that the two genes are not linked. Asci containing 2 pink:2 white; 0 pink:4 white; 1 pink:3 white, correspond to the same three categories and reveal that pink is produced as a result of a two-factor interaction.

Six asci from a pink by pink hybrid produced four pink cultures from each ascus. Three asci dissected from a homozygous adenine-dependent, methionine-dependent hybrid produced only white cultures. Seven asci from an ad(P)MET × AD MET back-cross hybrid produced 2 pink and 2 white per ascus. This further confirms the regular segregation of the two genes.

Adenine-dependence is the effect of the action of a single gene; pink pigment is a correlated effect which depends on the synergistic effect of

other genes as well. Pink pigment is apparently produced following the interaction of a precursor of adenine and an excess of methionine plus other substances. Pigment is usually produced in organisms incapable of completing the synthesis of adenine and capable of producing a considerable amount of methionine. The variation in intensity of color in different pink organisms indicates that many other factors affect color intensity.

False Mutations.—Some of the adenine-dependent, methionine-dependent *white* cultures were transferred to peptone agar to which an excess of methionine had been added. Pink cultures appeared thus confirming the dependence of the pink character on the presence of methionine. Added methionine did not induce the development of a pink color in any of the adenine-independent white organisms. When the cultures arising from homozygous ad met stocks were grown on agar, numerous small secondary pink papillae often appear suggesting local accumulation of sufficient methionine to produce the pink color.

Variations in bacteria following environmental changes have often been called "mutations" but the present experiments show that variations may also be due to a deficiency either of external or internal origin which prevents the development of the characteristic phenotype on a deficient medium. False "mutations" from pink to white may appear when growth occurs in the absence of sufficient methionine to insure the production of the pink color; many pink cultures have white borders which may arise when the supply of methionine in the medium becomes exhausted. Transfer of these false "mutants" to a medium containing sufficient methionine may result in a false "reverse mutation" from white to pink without any change occurring in the gene itself.

Depletion Mutation.—Tatum and Reaume discovered that white (ad MET) variant colonies which retain their methionine synthesizing ability often arise from pink cultures on vegetative propagation—a fact which we have confirmed, and which they will discuss in greater detail elsewhere. Numerous white variants of the original pink have appeared. These white variants are ad MET like the white variants of Tatum and Reaume. Similar white segregants arising in the pink pedigree were subjected to genitical analysis.

Two hybrids of one of the exceptional adenine-deficient methionine-sufficient (ad MET) *white* cultures (obtained among the 46 asci from the first hybrid in table 1) by standard pinks, ad(P)MET, produced 21 asci containing 4 pink cultures each. Eight asci from a hybrid of the exceptional white (ad MET) by a standard adenine-dependent, methionine-dependent white produced two pink and two white cultures per ascus; the white cultures were methionine-dependent. Six asci of an exceptional white (ad MET) by an adenine-independent, methionine-dependent white

produced the progeny that would be expected if the exceptional white were a normal pink. This analysis indicates that the inability of these exceptional adenine-dependent, methionine-independent cultures to produce the pink pigment was due to some mechanism which is restored to activity following hybridization.

The following hypothesis is invoked to explain the effect of outcrossing in restoring the pink color. Pink depends upon the presence of the two genes *ad* and *MET* plus some other substances (*X*, *Y*, *Z*, etc.). The substance *X* is an essential component of gene *X* which has no other components besides *X*. Continuous production of pink exhausts the supply of *X* and results in the "running out" of the character. The stock to which the outcross is made carries gene *X* with an intact supply of the *X* component for since the stock does not produce pink it does exhaust its supply of the *X* substance. The outcross automatically restores the *X* substance and reëstablishes the pink color. Other stocks may become white because *Y* or *Z* substances are exhausted. Mutations from pink to white are not the result of a drastic change in genotype but merely the result of the exhaustion of some gene component easily supplied by outcrossing to any normal stock.

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*WIND-DRIVEN CURRENTS IN A BAROCLINIC OCEAN; WITH APPLICATION TO THE EQUATORIAL CURRENTS OF THE EASTERN PACIFIC**

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1. *Introduction.*—Permanent ocean currents are computed from the observed distribution of density on the assumptions (1) that the horizontal pressure gradient is balanced by the Coriolis force (the deflecting force of the earth's rotation) and (2) that the horizontal velocities and the hori-